PHYSIOLOGY

Effect of Asphyxia on Adenylate Cyclase Activity in Cat Brain Cortex

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Effect of 1-5-min asphyxia on adenylate cyclase activity in cat brain cortex is studied. Adenylate cyclase activity is measured in cortex specimens obtained *ex vivo* after 1, 2.5, and 5 min of asphyxia, and 30 and 60 min of reoxygenation by radioassay. Stimulating effects of norepinephrine and NaF on adenylate cyclase activity are assessed. Five-min asphyxia induces phasic changes in adenylate cyclase activity: on the 1st min basal activity of the enzyme increases by 97%, after 2.5 min it returns to the initial level, and increases again by 55% on the 5th min of asphyxia. On the 30th and 60th min of reoxygenation after 2.5- and 5-min asphyxia, basal adenylate cyclase activity does not differ from the initial activity. The stimulating effect of norepinephrine and NaF on enzyme activity is weakened after 5 min of asphyxia and 30 min of reoxygenation after 2.5- and 5-min asphyxia. Even short-term asphyxia affects adenylate cyclase activity and modifies the mechanisms of adrenergic signal transduction in the brain cortex in response to oxygen deficiency and probably to hypercapnia as well as during the early reoxygenation period.

Key Words: *asphyxia; adenylate cyclase; brain cortex*

The membrane-bound enzyme adenylate cyclase (AC, EC 4.6.1.1) is an important component of the cAMP regulatory system. Adenylate cyclase mediates the effect of neurotransmitters and hormones (catecholamines, acetylcholine, opioids, etc.) and is a key component of the signal transduction system. The enzyme represents a transmembrane complex consisting of guanyl-binding regulatory subunit and catalytic subunit. Endogenous regulation of AC is modified predominantly through activation or inhibition of receptors coupled to the enzyme via Gs- or Gi-proteins, respectively, or through interaction with Ca^{2+}-calmodulin.

It is known that hypoxia disturbs synthesis and secretion of many neurotransmitters, including catecholamines [6]. Hypoxia-induced impairment of the synthesis and release of neurotransmitters plays an important role in disturbances of synaptic transduction both in response to oxygen deficiency and during the reoxygenation period. Moreover, an important role in the regulation of synaptic transduction is played by cAMP [5,14]. We have previously showed that 1-5-min asphyxia leads to marked phasic shifts in cAMP content in cat brain cortex during asphyxia and subsequent reoxygenation [1]. For evaluation of the mechanisms involving the cyclic adenosine monophosphate regulatory system into the brain cortex reactions to hypoxia and reoxygenation we studied the dynamics of AC activity in the brain cortex during 5-min asphyxia and early reoxygenation period.

MATERIALS AND METHODS

Experiments were carried out on 32 mature male cats under light Nembutal narcosis, immobilized with tubo-

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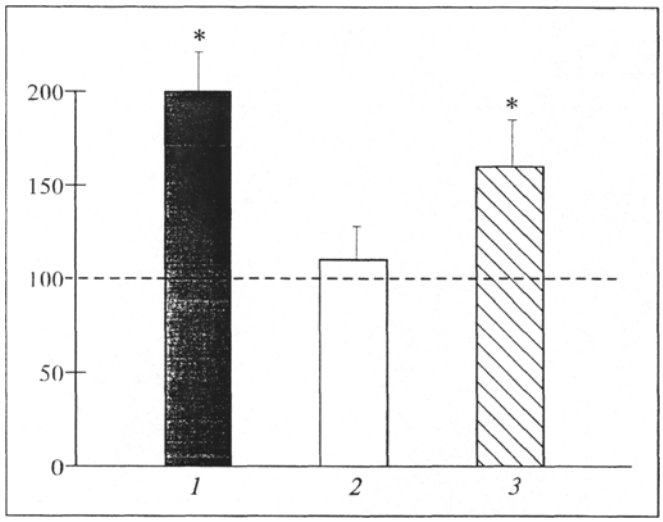

Fig. 1. Dynamics of adenylate cyclase activity during 5-min asphyxia. *1*) 1-min asphyxia (n=4); 2) 2.5-min asphyxia (n=6); 3) 5-min asphyxia (n=15). Here and in Fig. 2: ordinate: changes in AC activity, % of initial level (100%); *p<0.05 in comparison with the initial level (before asphyxia).

curarine, and artificially ventilated using a UIDZh-1 jet. Cranial trepanation was performed in order to obtain cortex specimens *ex vivo* for biochemical analysis. Asphyxia was modeled by turning off the ventilation jet for 2.5 and 5 min. The specimens were obtained during the normoxia period, 1, 2.5, and 5 min after cessation of ventilation, and after 30 and 60 min of subsequent reoxygenation period. Brain samples (10-20 mg) were dissected from the visual and sensorimotor cortex, frozen in liquid nitrogen, and stored at -80°C prior to assay.

Adenylate cyclase activity was measured using a radioisotope method. Product of the reaction (8-^{3}H×cAMP) was purified by successive chromatography on a Dowex 50×4 ion-exchange resin and alumina neutral [13]. Norepinephrine (NE) was used as AC activator, since it is a predominant catecholaminergic neurotransmitter in the studied brain areas in cats [4]. Sodium fluoride was used for determination of Gs-protein activity, since it stimulates dissociation of Gs-protein on subunits. One of them, GTP-binding α_s subunit, activates the catalytic subunit of AC [8]. The sample was homogenized in a 30-fold volume of 0.1 M Tris-HCl buffer, pH 7.6, containing 2 mM EDTA and 4 mM methylisobutylxanthine (MIX). Membrane preparation was obtained by centrifugation at 12,000 rpm for 20 min in a K-24 centrifuge with a refrigerator. The pellet was suspended in the same volume of buffer for homogenization and used for AC activity assay. The enzyme activity was assessed by production of 8-^{3}H×cAMP after incubation of the membrane preparation in the medium containing (in mM): 40 Tris-HCl (pH 7.6), 12 creatine phosphate, 5 magnesium acetate, 0.5 ATP, 2 cAMP, 1 dithiothreitol, 0.1 GTP, 1.6 MIX, 50 U/ml creatine phosphokinase, 0.1 mg/ml bovine serum albumin, and 2.2×10^6 cpm/sample 8-^{3}H×AMP. Norepinephrine and NaF were added to the incubation medium in concentrations of 10^{-5} and 10^{-3} M, respectively. The enzymatic reaction was carried out at 37°C for 10 min. The reaction was stopped by adding 0.1 ml stopping solution (2% sodium lauryl sulfate, 45 mM ATP and 1.3 mM cAMP) followed by 1.5-min boiling. Each experiment included several controls for evaluation of the efficiency of 8-^{3}H×cAMP isolation. To this end in the first case the stopping solution was added to the reaction mixture prior to the membrane preparation, in the second case the membrane preparation was replaced with an equivalent volume of buffer. Chromatography and recording of the elution profile for 8-^{3}H×ATP and 8-^{3}H×cAMP on Dowex 50×4 and Al_2O_3 columns were carried out as described elsewhere [13]. Radioactivity was measured in a Mark III counter (Tracor Europe). Protein content was determined by the method of Lowry. The data were processed statistically using the Student's t test.

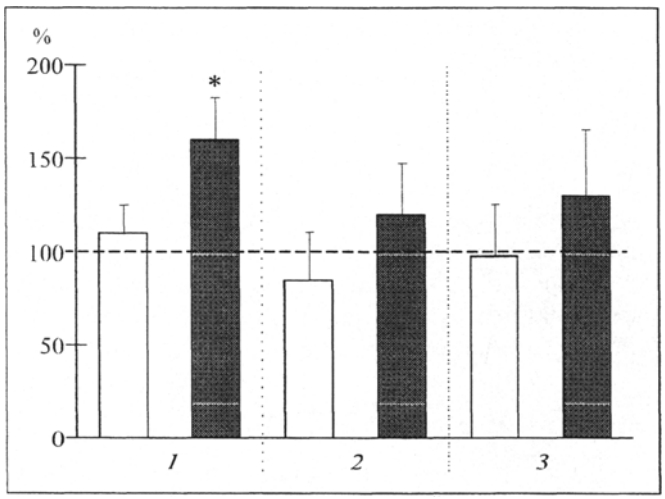

Fig. 2. Dynamics of adenylate cyclase activity during reoxygenation period after 2.5- and 5-min asphyxia (shown by light and dark bars, respectively). *1*) asphyxia; 2 and 3) 30th and 60th min of reoxygenation, respectively.

RESULTS

Basal AC activity and its sensitivity to NE and NaF in normoxia were similar in visual and sensorimotor cortex. Basal AC activity was 46.5±5.19 pmol cAMP/mg protein/min. Norepinephrine and NaF stimulated basal activity of the enzyme by 54 and 196%, respectively. Five-min asphyxia induced phasic changes in AC activity (Fig. 1). Stimulation of AC activity on the 1th min of asphyxia may result from accumulation of catecholamine receptor agonists [7].

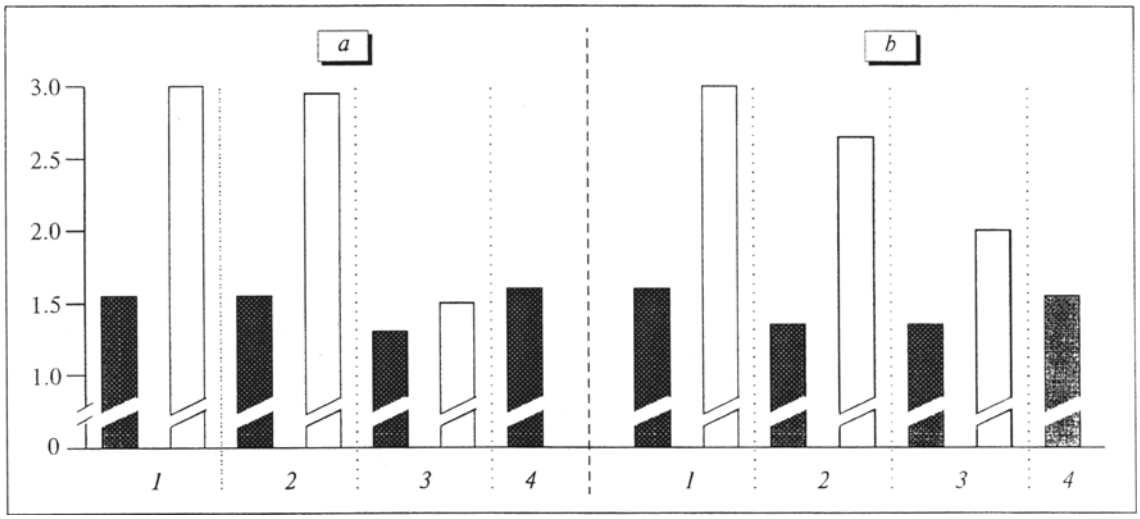

Fig. 3. Changes in adenylate cyclase sensitivity to NE (dark bars) and sodium fluoride (light bars) after 2.5- (*a*) and 5-min (*b*) asphyxia and during reoxygenation. Sensitivity under conditions of normoxia (*1*), asphyxia (*2*), and on 30th (*3*) and 60th (*4*) min of reoxygenation. Ordinate: changes in stimulated enzyme activity, rel. units. Basal adenylate cyclase activity at the correspondent stage of the experiment is taken as one unit.

cumulation of catecholamine receptor agonists [7]. Other possible mechanism is activation of AC by the Ca^{2+}-calmodulin complex [11], since shifts in cell Ca^{2+} content observed after 1 min of asphyxia [3,9] can induce this process. Adenosine considerably contributes to activation of AC on min 5 of asphyxia [12]; this transmitter is actively accumulated in response to neuron depolarization [10] occurring at this stage of asphyxia. Basal AC activity on the 30th and 60th min of reoxygenation after 2.5- and 5-min asphyxia did not differ from the baseline values (Fig. 2). On the other hand, we have shown that 2.5- and 5-min asphyxia markedly change the content of cAMP measured on min 30-60 of reoxygenation [1]. These data suggest that the observed shifts in the content of cAMP during reoxygenation after 2.5-5-min asphyxia result predominantly from modulation of phosphodiesterase activity.

No changes in NE- and NaF-stimulated AC activity were noted after a 2.5-min asphyxia (Fig. 3, *a*), whereas on min 30 of reoxygenation the sensitivity of the enzyme to NE and NaF stimulation decreased. On min 60 of reoxygenation the sensitivity of AC to NE recovered.

In contrast to 2.5-min asphyxia, 5-min asphyxia reduced the stimulating effect of NE and NaF on AC activity (Fig. 3, *b*). The decrease of AC sensitivity to NE and NaF after 30 min of reoxygenation was more pronounced in comparison with that observed after 2.5-min asphyxia. On min 60 of reoxygenation, the sensitivity of AC to NE was restored. The reduced sensitivity of the enzyme to NE probably attests to a reduced affinity of the receptor—Gs-protein—AC complex to β-adrenoreceptor agonists in the brain cortex due to oxygen deficiency and probably hypercapnia. A similar decrease in the stimulating effect of NE on AC was also noted in dog cortex after a 15-min ischemia [2].

The data on reduced stimulating effect of NaF on AC on min 5 of asphyxia and on min 30 of reoxygenation suggest that asphyxia induces long-term disturbances in Gs protein responsible for the β-adrenoreceptor—AC coupling [8]. A similar inhibition of Gs protein was demonstrated in the myocardium during ischemia [15].

These findings show that even relatively short-term (5 min) asphyxia changes activity of the AC-complex and modifies the mechanisms of adrenergic signal transduction in the brain cortex both in response to oxygen deficiency and, probably, hypercapnia, and in the early reoxygenation period.

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REFERENCES

1. E. A. Bolekhan, *Byull. Eksp. Biol. Med.*, **113**, No. 2, 227-228 (1992).
2. S. I. Pylova, *Neirokhimiya*, **7**, 39-46 (1988).
3. M. O. Samoilov, D. G. Semenov, N. G. Yarantsev, and S. A. Evdokimov, *Fiziol. Zh. SSSR*, **68**, 3-8 (1982).
4. N. S. Ahn and M. H. Makman, *Brain Res.*, **38**, 125-135 (1977).
5. R. P. Ebstein, K. Seamon, C. R. Creveling, and J. W. Daly, *Cell. Mol. Neurobiol.*, **2**, 179-192 (1982).
6. K. Gordon, J. B. Becker, and F. S. Silverstein, *J. Neurochem.*, **54**, 605-611 (1990).
7. R. A. Gross and J. A. Ferrendelli, *Ibid.*, **34**, 1309-1318 (1980).
8. B. P. Hughes and G. J. Barritt, *Biochem. J.*, **245**, 41-47 (1987).
9. J. W. Lasarewicz, M. O. Samoilov, and D. G. Semenov, *Resuscitation*, **15**, 245-255 (1987).

10. H. G. E. Lloyd, I. Spense, and G. A. R. Johnston, *Brain Res.*, **462**, 391-395 (1988).
11. M. T. Piasik, P. L. Wisler, C. L. Jonson, and J. D. Potter, *J. Biol. Chem.*, **255**, 4176-4181 (1980).
12. J. P. Robinson and D. A. Kendall, *J. Neurochem.*, **52**, 690-698 (1989).
13. Y. Salomon, in: *Advances in Cyclic Nucleotide Research*. G. Brocker *et al.* (Eds.), Vol. 10, New York (1979), pp. 35-55.
14. H. Shuntoh, K. Taniyama, H. Fukuzaki, and C. Tanaka, *J. Neurochem.*, **51**, 1565-1572 (1988).
15. E. Susunni, W. T. Manders, D. R. Knight, *et al.*, *Circ. Res.*, **65**, 1145-1150 (1989).

Effect of KLN-93 on Ventricular Fibrillation Induced by Reperfusion and Electrical Stimulation in Cats

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In experiments devoted to modeling of reperfusion ventricular fibrillation and determination of the electric threshold of fibrillation, a protective effect of KLN-93 (a para-aminobenzoic acid ester derivative) is compared with that of lidocaine. It is shown that KLN-93 in doses stopping reperfusion fibrillation is 2-4-fold less toxic than the isoeffective doses of lidocaine. In a dose of 0,4 mg/kg (2.5% LD_{50}) KLN-93 increases the fibrillation threshold 4.5-fold, while isotoxic dose of lidocaine increases this parameters approximately 2-fold. These data suggest that KLN-93 is an effective antifibrillatory agent.

Key Words: *antifibrillatory agents; lidocaine; crystallographycally homogeneous substances; conformers of local anesthetics*

Previous studies showed that crystallographycally homogeneous substance of the para-aminobenzoic acid ester derivative KLN-93 elicits a more potent antiarrhythmic effect in coronary arrhythmias in dogs and cats than isoeffective doses of lidocaine [4].

Similar results were obtained in aconitine-induced arrhythmia in rats and barium chloride-induced arrhythmia in rabbits [4]. Taking into account the lower toxicity of isoeffective doses of KLN-93 in comparison with lidocaine and its prolonged (to 40 min) effect after intravenous administration in arrhythmias reproduced as described previously [3], it seems very important to test KLN-93 as a potential antifibrillatory agent.

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MATERIALS AND METHODS

Acute experiments were carried out on 93 narcotized cats. Quantitative parameters of different experimental series are listed in Tables 1 and 2.

Early occlusion and reperfusion arrhythmias in narcotized cats were induced by intraperitoneal injection of 40 mg/kg Nembutal. Temporary occlusion of the anterior interventricular branch of the left coronary artery was made at the level of the lower edge of the auricle. The ligature was removed after 30 min, which induced reperfusion arrhythmia usually transformed into ventricular fibrillation (VF). KLN-93 (0.125% solution) and a reference agent lidocaine (1% solution, Egis, Hungary) were injected intravenously slowly in isotoxic doses constituting 1, 2.5, 5, 10, and 15% of LD_{50} (for rats upon intravenous administration) 5-7 min before coronary occlusion.

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